phbA; PHA synthase (phaC) and phasin (phaP); phaP and phaC [(1D)]; phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

4. (Amended) The fusion of claim 1 wherein the linker is comprised of glycine-serine.

Remarks

Claims 1-6 are pending. Claims 1, 2, and 4 have been amended. The Applicants have resubmitted PTO Form-1449 and copies of the documents cited therein (mailed on April 5, 2002). The present invention is directed to the construction and expression of fusion enzymes for the production of polymer, where the enzymes are specific bacterial enzymes, and the polymer is polyhydroxyalkanoate. The examples disclose the fusion of multimeric enzymes requiring the use of cofactors and which interact to synthesize polymer (page 5, lines 21-23).

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 1-6 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Claim 1 has been amended per the Examiner's suggestion to reference a single promoter driving expression of the fusion protein. Claims 1 and 2 have been amended to provide consistency with the use of the term "β-ketothiolase". The term "(1D)" has been deleted from claim 2. Claim 4 has been amended clarify the linker by including "comprising" language.

Rejection Under 35 U.S.C. § 102

Claims 1-3, 5, and 6 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,143,952 to Srienc *et al.* ("Srienc"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended. Enclosed is a Declaration under 37 C.F.R. 1.131, signed by all of the inventors prior to the effective date of the Srienc reference (March 31, 1998). The Declaration will be signed by all inventors and submitted shortly. The Declaration and accompanying notebook pages demonstrate (1) the prior use of specific DNA primers to sequence the claimed protein fusions (page 16); (2) protein fusion production of polymer in *E. coli* (page 88); (3) the high level production of polymer from the protein fusions (page 94); and (4) western blot analysis showing the correctly expressed fusion protein (page 96).

In addition to the submitted declaration and notebook pages, the Applicants respectfully submit that Srienc does not teach a protein fusion under the control of a single promoter. Srienc discloses a promoter in Figure 3. However, one of ordinary skill in the art will realize that this promoter drives transcription of an operon in which the genes of the operon are separately translated, thereby forming separate enzymes (see figures 2-4 of Srienc). Contrary to the examiner's assertion that column 24 of Srienc teaches that a fusion enzyme can be expressed in *E.coli*, the Applicant's respectfully submit that Srienc teaches the *screening* for a multienzyme complex without ever showing that such a complex *could* exist *in vivo* (this is the purpose of the proposed screen – to determine if one could exist). The disclosed bi- or multi-functional enzyme complex taught at column 7 of Srienc harbors a bi-specific polymerase with extended substrate specificity (bi-specific). While Srienc does teach a fusion protein at column 7, between a bi-

specific polymerase and one or more additional enzymes involved in the PHA pathway, Srienc does not teach a *fusion* protein (i.e. one ribosome binding site) under the control of a single promoter. Thus, Srienc is not enabling for transcriptional and translational sequences upstream of a first open reading frame that direct the synthesis of a single protein with the primary structure that comprises both original open reading frames. Furthermore, Srienc does not teach a two enzyme fusion, wherein the enzymes catalyze *successive reactions*.

Rejection Under 35 U.S.C. § 103

Claim 4 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 6,143,952 to Srienc *et al.* ("Srienc") in view of *Trends Biotech* 9:226-231, (1991) by Bulow *et al.* ("Bulow"), and *J. Mol. Biol.* 211:943-958, (1990) by Argos ("Argos"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Srienc

As discussed in the foregoing section, the Applicants have enclosed an executed Declaration under 37 C.F.R. 1.131 establishing invention of the claimed protein fusions prior to the effective date of the Srienc reference (March 31, 1998).

As discussed in the foregoing section under 35 U.S.C. § 102, Srienc does not teach a two enzyme fusion, wherein the enzymes catalyze *successive reactions*. Furthermore, while Srienc does teach a fusion protein at column 7, between a bi-specific polymerase and one or more additional enzymes involved in the PHA pathway, Srienc does not teach a *fusion* protein (i.e. one ribosome binding site) under the control of a single promoter. Thus, Srienc is not enabling for transcriptional and translational sequences upstream of a first open reading frame that direct the

synthesis of a single protein with the primary structure that comprises both original open reading frames.

Bulow

The Examiner relies upon Bulow for the teaching of short peptide linkers used to fuse enzymes (page 5 of Office Action mailed on February 27, 2002).

Argos

Argos is relied upon for a teaching of amino acid preferences as disclosed at page 203 of the reference. However, Argos admits that "further experiments are required to determine the most important of the various linker characteristics; namely, linker length, composition, sequence, geometry and the nature of the genes fused" (page 956, last column).

Summary

In summary, Srienc teaches a single promoter driving expression of three individually translated genes; an operon (Figures 2-4 of Srienc). Srienc discloses the *possibility* of generating protein fusions, without providing for a construct to produce such a fusion protein (see column 7). Srienc teaches a system to *screen* for a multienzyme complex comprising both reductase and polymerase activity, without having provided for the construction of a genetic vehicle to express such a complex. Therefore, the "gaps" in the logic of Srienc to produce a gene fusion between the enzymes claimed that is expressed *in vivo*, and wherein the gene fusion product retains its activity, are required to be addressed and "filled in" by Argos and Bulow.

Based upon the above discussion regarding Srienc, the Examiner is respectfully reminded that wholly prophetic statements are not a proper basis for invalidation of patent claims on grounds of anticipation.

> Prophetical suggestions and surmises in prior patents or publications of what results can be achieved in a particular art are not enough to negative novelty of any patent on an invention which can accomplish that result. [Citing to the decision of Learned Hand in *Dewey and Almy v Mimex Co*, 124 Fed. 2d 986, 989 (2d Cir. 1942).]

LIPSCOMB'S WALKER ON PATENTS, §4:27, page 358 (1984).

Argos fails to teach a genetic linker that when used in a construct to fuse any of the enzymes selected from the group listed in presently pending claim 1, would not hinder the expression of the two catalytically active enzymes selected. Argos does not address the use of a single promoter to drive expression of a single protein fusion that harbors successive enzymatic reactions.

Bulow teaches optimal length linkers, for the enzymes described therein, based upon the correct folding and accessibility of active sites in the recombinant enzymes.

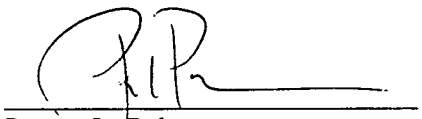
Therefore, a disclosure that is prophetic with regard to protein fusions between enzymes involved in PHA sythesis (Srienc), in combination with references that teach the use of various linker sizes and compositions (Argos and Bulow) do not provide for an enabling disclosure for successfully obtaining a protein that is comprised of two separate enzymes fused together, expressed from a single promoter, and is enzymatically active, catalyzing successive reactions, once translated.

The Examiner is respectfully reminded that references relied upon to support a rejection under 35 USC 103 must provide an enabling disclosure, i.e., they must place the claimed

invention in the possession of the public." *Application of Payne*, 606 F.2d 303, 314, 203 U.S.P.Q. 245 (C.C.P.A. 1979); *see Beckman Instruments, Inc. v. LKB Produkter AB*, 892 F.2d 1547, 13 U.S.P.Q.2d 1301 (Fed. Cir. 1989). A publication that is insufficient as a matter of law to constitute an enabling reference may still be relied upon, but only for what it discloses. *See Reading & Bates Constr. Co. v. Baker Energy Resources Corp.*, 748 F.2d 645, 651-652, 223 U.S.P.Q. 1168 (Fed. Cir. 1984); *Symbol Technologies, Inc. v. Opticon, Inc.*, 935 F.2d 1569 (Fed. Cir. 1991).

Allowance of claims 1-6 is respectfully solicited.

Respectfully submitted,



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Certificate of Mailing Under 37 C.F.R. § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Aisha Wyatt

Date: June 27, 2002

Clean Version of Amended Claims
Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Four times amended) A protein fusion having a formula selected from the group consisting of E1-L_n-E2 and E2-L_n-E1, wherein E1 and E2 catalyze successive reactions in a polyhydroxyalkanoate biosynthetic pathway and are each selected from the group consisting of β-ketothiolases, acyl-CoA reductases, polyhydroxyalkanoate synthases, poly(3-hydroxybutyrate) synthases, phasins, enoyl-CoA hydratases, and beta-hydroxyacyl-ACP::coenzyme-A transferase, in which linker L_n is a peptide of n amino acids that link E1 to E2 or E2 to E1, and wherein expression of the fusion protein is under the control of a single promoter resulting in expression of both catalytically active E1 and E2.

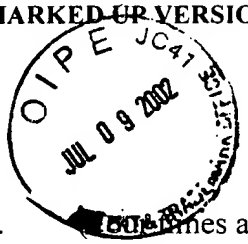
2. (Twice amended) The fusion of claim 1 wherein E1 and E2 are selected from the group consisting of β-ketothiolase (phbA) and acyl-CoA reductase (phbB); phbB and phbA; PHA synthase (phaC) and phasin (phaP); phaP and phaC; phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

3. (Unamended) The fusion of claim 1 wherein n in the linker is between zero and 50 amino acids.

4. (Amended) The fusion of claim 1 wherein the linker is comprised of glycine-serine.

5. (Unamended) The fusion of claim 1 expressed in a plant.

6. (Unamended) The fusion of claim 1 expressed in a bacteria.



COPY OF PAPERS ORIGINALLY FILED

Marked Up Version of Amended Claims

Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

1. (Four times amended) A protein fusion having a formula selected from the group consisting of E1-L_n-E2 and E2-L_n-E1, wherein E1 and E2 catalyze successive reactions in a polyhydroxyalkanoate biosynthetic pathway and are each selected from the group consisting of β-ketothiolases, acyl-CoA reductases, polyhydroxyalkanoate synthases, poly(3-hydroxybutyrate) synthases, phasins, enoyl-CoA hydratases, and beta-hydroxyacyl-ACP::coenzyme-A transferase, in which linker L_n is a peptide of n amino acids that link E1 to E2 or E2 to E1, and wherein [the] expression of the fusion protein is under the control of a single promoter resulting in expression of both catalytically active E1 and E2.

2. (Twice amended) The fusion of claim 1 wherein E1 and E2 are selected from the group consisting of [beta] β-ketothiolase (phbA) and acyl-CoA reductase (phbB); phbB and phbA; PHA synthase (phaC) and phasin (phaP); phaP and phaC [(1D)]; phaC and beta-hydroxyacyl-ACP::coenzyme-A transferase (phbG); phbG and phaC; phaC and enoyl-CoA hydratases (phaJ); and phaJ and phaC.

3. (Unamended) The fusion of claim 1 wherein n in the linker is between zero and 50 amino acids.

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